DP Barcode: D215429 MRID No.: 436200-01

DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE FISH
§ 72-3(A)

1. **CHEMICAL:** Didecyl Dimethyl Ammonium Chloride (DDAC) PC Code No.: 069149
2. **TEST MATERIAL:** DDAC Purity: 80.5%
   ^{14}C-DDAC 84.6 radiochemical purity
3. **CITATION**
   Authors: Maura K. Collins
   Title: Didecyldimethylammoniumchloride (DDAC): Evaluation in a Static Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*)
   Study Completion Date: March 15, 1994
   Laboratory: Springborn Laboratories, Inc., Wareham, MA
   Sponsor: Lonza, Inc., Fair Lawn, NJ
   Laboratory Report ID: 93-6-4833
   MRID No.: 436200-01
   DP Barcode: D215249
4. **REVIEWED BY:** Christian M. Newman, Wildlife Biologist
   KBN Engineering and Applied Sciences, Inc.
   Signature: [signature] **Date:** 11/2/95
   APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist
   KBN Engineering and Applied Sciences, Inc.
   Signature: P. Kosalwat **Date:** 11/2/95
5. **APPROVED BY:** ~~Ann Stavola~~, Head, Section ~~5~~, EFED
   Signature: HENRY T. CRAVEN 6/21/94 **Date:** 2-16-96
6. **STUDY PARAMETERS**

   Age or Size of Test Organism: 0.30 g
   Definitive Test Duration: 96 hours
   Study Method: Static-renewal
   Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study is scientifically valid and meets the guideline requirements for a static acute toxicity test using sheepshead minnows (*Cyprinodon variegatus*). The LC_{50} of 0.96 ppm mean meausured concentration classifies DDAC as highly toxic to sheepshead minnow. The NOEC was determined to be 0.39 ppm mean measured concentration.

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Results Synopsis

LC_{50}: 0.96 ppm ai 95% C.I.: 0.77-1.17 ppm ai
NOEL: 0.39 ppm ai Probit Slope: 9.13

8. ADEQUACY OF THE STUDY

A. Classification: Core.

B. Rationale: N/A

C. Repairability: N/A.

9. Guideline Deviations

1. The test organisms had a mean weight of 0.30 g (range of 0.19-0.39 g); the guidelines recommend 0.5-5 g.
2. Test organisms were not fed 24 hours prior to testing instead of the recommended 48 hours.
3. Dissolved oxygen concentration in two test chambers was <60% of saturation at 48 hours.

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the sheepshead minnow (*Cyprinodon variegatus*) or the Silverside (*Menidia sp.*).	*Cyprinodon variegatus*
Mean Weight 0.5 - 5 g	0.30 g
Mean Standard Length Longest not > 2x shortest	Mean: 28 mm Range: 22-35 mm
Supplier	Aquatic Biosystems, Fort Collins, CO
All fish from same source?	Yes
All fish from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 14 days	21 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Not reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study	The fish were not fed 24 hours prior to test initiation or during the test period.
Pretest Mortality <3% mortality 48 hours prior to testing	0% mortality 48 hours prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Filtered seawater
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ salinity, weekly range < 6 ‰	30-32‰
Water Temperature 22 ± 1 °C	21-23°C; test temperature was maintained using a water bath.
pH 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8	7.7-8.1

Guideline Criteria	Reported Information
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	≥44% of saturation during the first 48 hours and ≥74% of saturation during the last 48 hours
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	1. glass 2. 18.9 liters 3. 15 liters
Type of Dilution System Must provide reproducible supply of toxicant	N/A
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.20 g/L
Photoperiod 16 hours light, 8 hours dark	16 h light, 8 h dark.
Solvents Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	None was used.

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	After 96 hours, 40% mortality at 10 mg/L treatment level and no mortality at 0.10, 0.50, and 5.0 mg/L.

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	Control and 0.39, 0.69, 1.2, 1.9, and 3.1 ppm ai
Number of Test Organisms Minimum 10/level, may be divided among containers	10 fish per aquarium; 1 aquarium per treatment or control.
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes; biological observations were made at 3, 6, 24, 48, 72, and 96 hours.
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	1. Temperature was measured continuously in the water bath and daily in test solutions. 2. DO and pH were measured daily in each test solution.
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	New test solutions were measured at 0 and 48 hours, and old test solutions were measured at 96 hours.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	Mean recovery was 100-109%
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0%
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal (ppm ai)	Mean Measured (ppm ai)		24	48	72	96
Control	-	10	0	0	0	0
Solvent Control	-	-	-	-	-	-
0.39	0.39	10	0	0	0	0
0.65	0.69	10	0	0	0	1
1.10	1.20	10	0	6	6	8
1.80	1.90	10	10	10	10	10
3.0	3.10	10	10	10	10	10

Other Significant Results: All surviving minnows exhibited darkened pigmentation and lethargy after 48 hours at the 1.2 ppm ai treatment level.

B. Statistical Results

Method: Moving average method

96-hr LC_{50}: 0.94 ppm 95% C.I.: 0.77-1.20 ppm ai

Probit Slope: N/A No mortality conc.: 0.39 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC_{50} (C.I.)	0.95 ppm ai
Moving Average Angle LC_{50} (95% C.I.)	0.93 (0.70-1.17) ppm ai
Probit LC_{50} (95% C.I.)	0.96 (0.77-1.17) ppm ai
Probit Slope	9.13
NOEC	0.39 ppm ai

14. REVIEWER'S COMMENTS: This study is scientifically sound and meets the guideline requirements for a static acute toxicity test using the sheepshead minnow (*Cyprinodon variegatus*). The LC_{50} of 0.96 ppm mean measured concentration classifies DDAC as highly toxic to sheephead minnow. The NOEC is 0.39 ppm ai. This study can be classified as **CORE.**

CHRISTIAN NEWMAN DIDECYLDIMETHYLAMMONIUMCHLORIDE CYPRINODON VARIEGATUS
10-20-95

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3.1	10	10	100	9.765625E-02
1.9	10	10	100	9.765625E-02
1.2	10	8	80	5.46875
.69	10	1	10	1.074219
.39	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT .69 AND 1.9 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .9545905

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.1144044	.9302615	.6988069	1.173191

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.320284	1	.9972983

SLOPE = 9.125761
95 PERCENT CONFIDENCE LIMITS = 3.961161 AND 14.29036

LC50 = .960539
95 PERCENT CONFIDENCE LIMITS = .7741638 AND 1.171541

LC10 = .6971858
95 PERCENT CONFIDENCE LIMITS = .4190398 AND .8430448

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